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**Differentiation of human embryonic stem cells to HOXA+ hemogenic vasculature that resembles the aorta-gonad-mesonephros.**

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**Authors:** Elizabeth S Ng, Lisa Azzola, Freya F Bruveris, Vincenzo Calvanese, Belinda Phipson, Katerina Vlahos, Claire Hirst, Vanta J Jokubaitis, Qing C Yu, Jovana Maksimovic, Simone Liebscher, Vania Januar, Zhen Zhang, Brenda Williams, Aude Conscience, Jennifer Durnall, Steven Jackson, Magdaline Costa, David Elliott, David N Haylock, Susan K Nilsson, Richard Saffery, Katja Schenke-Layland, Alicia Oshlack, Hanna K A Mikkola, Edouard G Stanley, Andrew G Elefanty

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**Funding Grants:** A suite of engineered human pluripotent stem cell lines to facilitate the generation of hematopoietic stem cells

**Public Summary:**

**Scientific Abstract:**

The ability to generate hematopoietic stem cells from human pluripotent cells would enable many biomedical applications. We find that hematopoietic CD34<sup>+</sup> cells in spin embryoid bodies derived from human embryonic stem cells (hESCs) lack HOXA expression compared with repopulation-competent human cord blood CD34<sup>+</sup> cells, indicating incorrect mesoderm patterning. Using reporter hESC lines to track the endothelial (SOX17) to hematopoietic (RUNX1C) transition that occurs in development, we show that simultaneous modulation of WNT and ACTIVIN signaling yields CD34<sup>+</sup> hematopoietic cells with HOXA expression that more closely resembles that of cord blood. The cultures generate a network of aorta-like SOX17<sup>+</sup> vessels from which RUNX1C<sup>+</sup> blood cells emerge, similar to hematopoiesis in the aorta-gonad-mesonephros (AGM). Nascent CD34<sup>+</sup> hematopoietic cells and corresponding cells sorted from human AGM show similar expression of cell surface receptors, signaling molecules and transcription factors. Our findings provide an approach to mimic in vitro a key early stage in human hematopoiesis for the generation of AGM-derived hematopoietic lineages from hESCs.

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